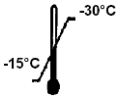
IMMUCOR

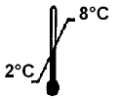
ATS-13® Activity Assay

REF ATS-13



Box A (303281)

RUO For Research Use Only. Not for use in diagnostic procedures.



Box B (303282)

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INTENDED USE

ATS-13 Activity Assay is for the quantitative measurement of ADAMTS-13 protease activity.

SUMMARY AND EXPLANATION

It was recently discovered that ADAMTS-13 is the protease responsible for cleaving von Willebrand Factor; deficiency of ADAMTS-13 activity has been demonstrated in the plasma of thrombotic thrombocytopenic purpura (TTP) patients. The lack of ADAMTS-13 activity results in the accumulation of multimers of von Willebrand Factor in the plasma and ultimately intravascular platelet aggregation resulting in the clinical symptoms associated with TTP.[4,5] Mild or moderately decreased levels of ADAMTS-13 activity have also been associated with other disease states and conditions.[2-5]

PRINCIPLE OF THE PROCEDURE

The ATS-13 Activity Assay is based on fluorescence resonance energy transfer (FRET) technology. A synthetic fragment of the von Willebrand Factor protein is used as the Substrate. Cleavage of this peptide between two modified residues releases the fluorescence quenching capabilities.

This assay is based on quantifying the cleavage of a small fragment of von Willebrand Factor by the ADAMTS-13 protease. The cleavage of this synthetic substrate is detected by reading the fluorescence that results when the substrate is cleaved.

REAGENTS

Maximum number of tests per kit:

- **ATS-13: 40 tests per kit**

All reagents should be stored as directed by the label.

	REF	
ATS-MS	403575	Black Microwell Strips: Once removed from the foil pouch, take care not to expose the strips to dust or particulates. Take care to protect from moisture. Strips should be stored at room temperature. Ready to use.
ATS-SUB	403614	Substrate: lyophilized. Keep substrate protected from light. Store lyophilized material at -15 to -30°C. Hydrated substrate should be stored upright in the original stoppered vial, sealed with Parafilm wrap, at -15 to -30°C (non-cycling freezer) in the dark.
ATS-SD	403600	Specimen Diluent: Ready for use. Store at 2 to 8°C.
ATS-SB	403612	Substrate Buffer: Ready for use. Store at 2 to 8°C.
PCH	403597	Positive Control: High: Store at -15 to -30 °C. Contains human source material. Thaw and mix thoroughly before use. Ready for use. Values can be found on ATS-13 Activity Assay Calibrator and Control Recording Sheet. Discard after single use.
PCL	403598	Positive Control: Low: Store at -15 to -30°C. Contains human source material. Thaw and mix thoroughly before use. Ready for use. Values can be found on ATS-13 Activity Assay Calibrator and Control Recording Sheet. Discard after single use.
ATS-CALA	403577	Calibrator A: Store at -15 to -30°C. Contains human source material. Thaw and mix thoroughly before use. Ready for use. Values can be found on ATS-13 Activity Assay Calibrator and Control Recording Sheet. Discard after single use.
ATS-CALB	403578	Calibrator B: Store at -15 to -30°C. Contains human source material. Thaw and mix thoroughly before use. Ready for use. Values can be found on ATS-13 Activity Assay Calibrator and Control Recording Sheet. Discard after single use.
ATS-CALC	403579	Calibrator C: Store at -15 to -30°C. Contains human source material. Thaw and mix thoroughly before use. Ready for use. Values can be found on ATS-13 Activity Assay Calibrator and Control Recording Sheet. Discard after single use.

ATS-CALD	403580	Calibrator D: Store at -15 to -30°C. Contains human source material. Thaw and mix thoroughly before use. Ready for use. Values can be found on ATS-13 Calibrator and Control Recording Sheet. Discard after single use.
ATS-CALE	403581	Calibrator E: Store at -15 to -30°C. Contains human source material. Thaw and mix thoroughly before use. Ready for use. Values can be found on ATS-13 Activity Assay Calibrator and Control Recording Sheet. Discard after single use.

PRECAUTIONS

- Do not use reagents that are turbid or contaminated.
- Care MUST be taken to avoid contamination of Calibrators and Substrate. Inadvertent contamination of these reagents with human plasma will invalidate the assigned values of the calibrators.
- Unopened and lyophilized reagents are stable until the expiration date printed on the box when stored as directed.
- Do not use reagents beyond their expiration date.
- Microwells and reagents contained in the kit are not to be used in conjunction with any other test system.
- Discard any unused portions of Calibrators, Controls, and used Black Microwell Strips after each run.
- Substitution of components other than those provided in this kit may lead to inconsistent or erroneous results.
- When making dilutions, follow pipette manufacturer's instructions for appropriate dispensing and rinsing techniques.
- The enzyme substrate reaction is temperature sensitive and should be performed in a controlled area at 22 to 25°C.
- Only plasma should be used in the assay. Serum will give inaccurate results.
- Care MUST be taken to avoid the introduction of particulate material (cardboard/paper towel fiber, foam debris, dust, etc.) into the wells of the assay

CAUTION

- All human plasma used in the Calibrators and Positive Controls for this product has been tested and found negative for antibody to HIV, HCV and HBsAg by FDA approved methods. No test method, however, can offer complete assurance that HIV, Hepatitis C virus, Hepatitis B virus or other infectious agents are absent. Therefore, these materials should be handled as potentially infectious.
- Discard all components when completed according to local regulations.

SPECIMEN COLLECTION AND STORAGE

Sample Collection and Preparation

NOTE: Only platelet poor plasma collected in 3.2% sodium citrate may be used for this assay. Do not use plasma that has been collected in or treated with EDTA. See Collection, Transport and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays. Approved Guideline H21-A4 NCCLS, Volume 23, Number 35, December 2003 for details.

Plasma collection should be performed as follows:

1. Collect blood in buffered sodium citrate (light blue top, 3.2%) plastic tubes (available in 4.5 mL, 2.7 mL or 1.8 mL full draw tubes).

NOTE: Partial draw tubes should NOT be processed. Since the tubes are pre-calibrated to draw the specified amount of blood, the resulting sample, will not have the proper 9:1 ratio of blood to anticoagulant if a full sample is not collected.

2. After collection, store tube upright at room temperature until centrifugation.

NOTE: Blood samples should be centrifuged between fifteen minutes and two hours after blood collection for best results.

3. Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times.

4. Centrifuge blood sample at room temperature in a horizontal rotor (swing-out rotor) for 15 - 20 minutes at 1500 to 1800 RCF (Relative Centrifugal Force) with the brake off.

   WARNING: Excessive centrifuge speed (over 2000 RCF) may cause tube breakage and exposure to blood and possible injury.

5. Following centrifugation, transfer the top 2/3 of the plasma layer into a new plastic tube.
6. Re-centrifuge the collected plasma at 1500 to 1800 RCF with the brake off for an additional 15 - 20 minutes to remove any red cells or platelets.
7. Transfer the top 2/3 of the plasma into a new plastic tube, taking care not to disturb any cells at the bottom of the tube.

Sample Storage

1. Plasma should be stored at 2 to 8°C and assayed within 4 hours OR aliquoted and frozen at -70°C or colder for up to 6 months.
2. Frozen plasma should be thawed rapidly at 37°C. Thawed plasma should be stored at 2 to 8°C and assayed within 4 hours.

PROCEDURE

Materials Provided

Box A (303281):
1. 6 x 130 µL Positive Control: High
2. 6 x 130 µL Positive Control: Low
3. 6 sets of Calibrators, 5 levels, 130 µL each:
   Calibrator A, Calibrator B, Calibrator C, Calibrator D, Calibrator E.
4. 1 x 0.10 mg Substrate

Box B (303282):
1. 2 Microwell frames, each containing 6 – 2 x 8 Black Microwell Strips
2. 1 x 14 mL Specimen Diluent
3. 1 x 14 mL Substrate Buffer

Additional Materials Required

1. Polypropylene plastic test tubes for patient sample dilutions and substrate dilution
2. Transfer pipets
3. Adjustable micropipets to deliver 10 - 100 µL and 100 – 1000 µL
4. Disposable tips
5. DMSO (Reagent Grade)
6. Fluorescent plate reader capable of measuring fluorescence at Excitation = 340 - 350 nm and Emission = 440 - 450 nm
7. Timer
8. Centrifuge
9. Aluminum Foil
10. CD (Compact Disc) (503001) (available from Immucor Customer Service)
    - ATS-13 Analysis Workbook
    - User Manual
11. Reporting Card (available at www.immucor.com)
12. Plate Layout Sheet, optional (available at www.immucor.com)

Test Procedure

1. Allow all reagents to warm to room temperature.

NOTE: Only remove one set of Calibrators and Controls per assay.

2. Determine the number of patient samples to be tested. Using the Recording Sheet, assign each sample to a location consisting of two (duplicate) wells.
   Record the identity of each sample on the Recording Sheet. Place the sample replicates horizontally (e.g. CALA in wells A1 and A2).

3. Remove microwell frame from pouch. Promptly remove unneeded strips from frame and reseal in the protective pouch.

4. In a plastic test tube, dilute each patient plasma sample to be tested by adding 18 µL plasma into 132 µL Specimen Diluent.

5. Add 50 µL of each Calibrator (in duplicate) to the appropriate microwells of the black microwell strips as designated on the Recording Sheet. Do not dilute.

6. Add 50 µL of Positive Control: Low (in duplicate) to the appropriate microwells of the black microwell strips as designated on the Recording Sheet. Do not dilute.

7. Add 50 µL of Positive Control: High (in duplicate) to the appropriate microwells of the black microwell strips as designated on the Recording Sheet. Do not dilute.

8. Add 50 µL of the prediluted sample plasma solution (prepared in step 4) in duplicate to the appropriate microwells of the black microwell strips as designated on the Recording Sheet.

NOTE: If multiple patient samples are tested at the same time, only one set of calibrators and controls are required.

9. Prepare Stock Substrate Solution. Remove stopper carefully as some Substrate may cling to the plastic. Reconstitute the lyophilized Substrate by adding 37 µL of reagent grade DMSO to the Substrate vial. Mix solution and add 113 µL reagent grade H_2O. Replace the stopper and close the cap tightly. Mix well by gently swirling until all contents are dissolved.

10. Prepare the assay Substrate Solution (3%) in a plastic tube according to the table below:

Patient Samples to Test	Volume Stock Substrate Solution (µL)	Volume Substrate Buffer (µL)
1	25	795
5	37	1193
10	53	1714
40*	150	4850

* This can be prepared by adding substrate buffer directly to stock substrate vial if being used for testing within one assay.

NOTE: A repeating pipette should not be used.

11. Mix the solution thoroughly. Protect from light. Immediately following preparation, add 50 µL of Substrate Solution into each microwell containing a patient sample, calibrator, or control. Gently tap the sides of the microwell frame to ensure even distribution.

NOTE: Remaining Stock Substrate Solution should be stoppered and stored upright in the original vial with original stopper (sealed with Parafilm wrap) at –20°C (non-cycling freezer) in the dark. Re-hydrated stock can be used for up to 6 months following re-hydration.

12. Place plate in fluorimeter with Excitation = 340 - 350 nm and Emission = 440 - 450 nm at room temperature. Read and start timer for 30 minutes. Record results as time zero.

NOTE: Reading must be taken within 5 minutes of addition of substrate.

13. Remove plate from fluorimeter, store plate at room temperature (not in plate reader) and protect from light.

NOTE: Do not cover plate with paper or cardboard. Fibers in the plate can cause random fluorescence. Cover with aluminum foil.

14. At 30 minutes, place plate in fluorimeter,with Excitation = 340 - 350 nm and Emission = 440 - 450 nm at room temperature. Read and record results.

CALCULATIONS AND RESULTS

Use the ATS-13 Analysis Workbook provided on the CD (503001) to obtain the results (% Normal ADAMTS-13 Activity) for controls and samples. Instructions for the workbook are provided in the User Manual included on the CD (compact disc).

The values calculated for the Positive Control: High and Positive Control: Low should fall within the allowable range identified on the lot specific ATS-13 Reporting Card. Assays where the controls do not meet these criteria should be considered invalid and should be repeated.

Plasma samples with calculated ADAMTS-13 activities greater than the value assigned for Cal E will be reported as greater than the assigned value for Cal E by the Analysis Workbook.

REFERENCES

1. "Collection, Transport and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays". *Approved Guideline H21-A4 NCCLS* 2003; **23(35)**.
2. Kokame K, Matsumoto M, Fujimura Y, Miyata T. *Blood* 2004; **103:**607.
3. Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. *Br. J. Haematol* 2005; **129:**93.
4. Lämmle B, George JN. *Seminars in Hematology* 2004; **41:**1:1.
5. Lämmle B, Kremer Hovinga JA, Alberio L. *J Thromb Haemost* 2005; **3:**1663.

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Warning	Warning
H302	Harmful if swallowed
H317	May cause an allergic skin reaction
H373	May cause damage to organs (Kidney) through prolonged or repeated exposure if swallowed.
P260	Do not breathe dust/fume/gas/mist/vapours/spray
P261	Avoid breathing dust/fume/gas/mist/vapours/spray
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product
P272	Contaminated work clothing should not be allowed out of the workplace
P280	Wear protective gloves/protective clothing/eye protection/face protection
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. Rinse mouth.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P314	Get medical advice/attention if you feel unwell.
P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P501	Dispose of contents/container to an approved waste disposal plant.